RESEARCH PAPER

Nephroprotective and Antidiabetic Effects of *Salvia hispanica* and *Nigella sativa* in Streptozotocin-Induced Diabetic Nephropathy Albino Wistar Rats

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ABSTRACT:

Diabetic nephropathy (DN) is still one of the leading causes of ESRD due to oxidative stress and inflammation resulting from chronic hyperglycemia. In the current study, the Nephroprotective and anti-diabetic effects of Salvia hispanica (chia) and Nigella sativa (kalonji) seed extracts were evaluated in STZ-induced diabetic albino Wistar rats. The animals were treated orally with chia and kalonji extracts at doses of 500, 750, and 1000 mg/kg for six weeks. The glycemic indices, renal function markers, oxidative stress parameters, and histopathological variations were analyzed. The diabetic control animals showed extreme hyperglycemia, increased HbA1c, impaired renal function (high BUN and creatinine, low albumin), augmented oxidative stress (low SOD, catalase, GSH; high MDA), and disastrous damage in renal tissue. The administration of chia and kalonji extracts significantly ameliorated all parameters in a dose-dependent manner. At the dose of 1000 mg/kg, the values for blood glucose, HbA1c, renal function parameters, and antioxidant enzymes almost reached normal values indicating improvement noticed in the histological analysis of the renal tissue. The maximum dosage achieved impacts similar to Losartan, a common Nephroprotective medication. This study suggests that chia and kalonji provide synergistic advantages because their content of polyphenols, omega-3 fatty acids, and thymoquinone all help enhance the body's antioxidant functioning and thus mitigate inflammatory processes and improve control over the body's glycemic levels. The study demonstrates kalonji and chias potential therapeutic application as additional medications. However, more clinical research needs to be conducted to confirm their significance in clinical practice with humans.

Keywords: Salvia Hispanica, Nigella Sativa, Diabetic Nephropathy, Nephroprotection, Antidiabetic.

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INTRODUCTION

Diabetic nephropathy is one of the most serious complications associated with diabetes mellitus, with signs of kidney damage manifesting as progressively elevated levels of albumin in the urine, declining rates of glomerular filtration (kidney function), and ultimately, advancing to stage 5 chronic kidney disease [1-7]. DN globally accounts for approximately 30%-40% of all chronic kidney disease members and is a leading cause of dialysis and kidney transplantation [8-11]. The impact of *Nigella Sativa* Oil on oxidative stress, inflammatory and glycemic control parameters in diabetic hemodialysis patients: a double-blind, randomized, controlled trial - PMC. Hyperglycemia causes irreversible structural and functional damage to the kidneys, including glomerular hypertrophy (enlarged kidneys), mesangial

expansion (swelling of cells within the glomerulus) and thickening of the basement membrane, compromising renal function and accelerating the rate of disease progression [12-24]. Despite advancements in treatment modalities, including using renin-angiotensin system inhibitors and SGLT-2 inhibitors, DN continues to be an important clinical challenge, and new approaches to treating this disease must be examined [25-28].

Oxidative stress and renal dysfunction

Oxidative stress is thought to be one of the main mechanisms by which DN occurs. Hyperglycemia leads to overproduction of reactive oxygen species (ROS) resulting in increased lipid peroxidation, protein oxidation, and DNA damage in the kidneys [29-34]. The imbalance of ROS with the body's antioxidant systems, which include superoxide dismutase (SOD), catalase and glutathione (GSH), increases glomerular injury and tubular injury [35-41]. Malondialdehyde (MDA) is one of the byproducts of lipid peroxidation that is consistently elevated in DN animal models, indicating oxidative stress as a therapeutic target for the treatment of DN [42-46]. Furthermore, oxidative stress interacts with inflammatory pathways to increase renal scarring and promote nephron loss [47-51].

Therapeutic potential of nutraceuticals

The health benefits of nutraceuticals, primarily plant-based, are gaining recognition for their anti-inflammatory, antioxidant and anti-diabetic properties [52, 53]. Certain dietary changes can produce changes in numerous pathways that are sensitive to nutritional factors, thereby reducing the effects of oxidative stress on the kidneys and improving outcomes in patients with kidney disease [54-56]. Two examples of seeds that are nutraceuticals valuable are *Salvia hispanica* (chia seed) and *Nigella sativa* (kalonji), which contain many bioactive compounds such as polyphenols, flavonoids, omega-3 fatty acids, and thymoquinone, all of which possess potent free radical scavenging capabilities and help regulate blood sugar levels [57-59]. As supplements, nutraceuticals have been shown to help lower HbA1c levels, improve antioxidant enzyme activity and restore kidney function markers in individuals with diabetes [60, 61].

Rationale for combining chia and kalonji

Other significant bioactive compounds found in chia seeds include alpha-linolenic acid, dietary fiber, and polyphenols, which aid in regulating blood sugar and lipid metabolism [62]. *Kalonji seeds* (Nigella sativa) contain thymoquinone, an antioxidant that has been shown to be anti-inflammatory and antioxidant [58]. Another synergistic effect occurs when both types of seeds are combined, acting on overlapping pathological pathways causing kidney injury (hyperglycemia, oxidative stress and inflammation). While chia seeds have been known primarily for their role in blood glucose metabolism and lipids, kalonji seeds are more efficacious to the body's antioxidant defense mechanisms and inhibit the increase of inflammatory mediators. Accordingly, the combination of the two seeds would have significant value as a nutraceutical treatment for complete kidney protection [63, 64].

Study objectives

The study evaluated the Nephroprotective and antidiabetic properties of salad (chia) and kalonji (*Nigella sativa*) seeds in a model of diabetic nephropathy induced by the administration of streptozocin in male albino Wistar rats. Diabetic nephropathy still accounts for a large percentage of end stage renal disease due to the oxidative stress in kidneys and is the most common cause of renal injury. Although pharmacotherapy of diabetes is currently treated with Nephroprotective agents such as renin-angiotensin system agents and SGLT2 inhibitors, there is an increasing interest in the use of nutraceutical agents as an adjunct to pharmacotherapy for diabetic nephropathy.

This research's primary objective is to evaluate the effect of kalonji (Nigella sativa) and chia (Salvia hispanica) seed extract on glycemic control, as measured by fasted blood glucose and HbA1c levels, in diabetic nephropathy rats. The secondary objective will be to evaluate kidney function markers (blood urea nitrogen, serum creatinine, and serum albumin) as indicators of kidney health.

A third aim of this research will be to evaluate the antioxidant defense mechanisms of the kidneys through measurement of superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and malondialdehyde (MDA) levels. Results will provide evidence of how oxidative stress mediator modulators may be used to develop treatment therapies for diabetic nephropathy. The last objective of this study is to corroborate the biochemical data by assessing the glomeruli and tubules of the renal tissue histologically for signs of structural repair. Ultimately, the goal of this research is to demonstrate the potential of using chia and kalonji seeds as a combined nutritional

supplement/alternative therapy for the treatment of diabetic nephropathy, and to establish a link between dietary and pharmacological management of diabetic nephropathy through the integration of biochemical, antioxidant, and histological analyses.

MATERIALS AND METHODS

Experimental animals

The laboratory Wistar male rats were housed within a registered animal facility according to institutional guidelines, from a source that met institutional standards for animal health and welfare. They were between 180–220 g, and they were all adults (indicating that they were mature), and are all albino (indicating that the rats were homogenous in regards to skin tone). The rats were transported to the laboratory under maintained controlled conditions/ light/dark cycle of 12 hours, temperature 22 ± 2 °C, and relative humidity of 50%-60% to create a suitable environment. The rats had free access to commercial pelleted food and water while housed in the laboratory [65]. Studies at the laboratory were conducted following guidelines from the CPCSEA of the Government of India, approved by an Institutional Animal Ethics Committee of the study (IAEC/04/02/2025). Furthermore, the studies adhered to all ethical principles concerning animal welfare to minimize animal suffering and instead promote the maximize replacement of animals with non-animal methods; the keeping of the number of animals used to a minimum; and changing experimental procedures in any possible manner to minimize pain/discomfort to the animal.

Induction of diabetic nephropathy (Streptozotocin model)

Diabetic nephropathy was induced in the diabetic rat model with Streptozotocin (STZ), which is a synthetic analogue of Insulin and acts as an analogue of Insulin (i.e., when STZ is injected into an animal, it produces high levels of glucose). Each rat received an intraperitoneal injection of 55 mg/kg of body weight (or one single injection) of freshly prepared solution of STZ dissolved in citrate buffer (pH 4.5). In order to confirm hyperglycemia in an animal, blood (serum) glucose levels were obtained from rats that were 72 hours fasted without the use of anesthetics. Hyperglycemia in an animal is defined as any serum glucose level which exceeds 250 mg/dL. Before determining progression of nephropathy, the degree of hyperglycemia and renal insufficiency was continuously observed for a 4-week period of time, which is consistent with previous studies. Streptozotocin is widely recognized for its ability to selectively destroy pancreatic β-cells and for its reproducibility of diabetic complications, such as nephropathy, in rodent animals [66, 67].

Treatment groups

Six groups of six albino Wistar rats were randomly assigned into six experimental groups, to provide a balanced distribution and minimize bias. The six groups consisted of **a)** a normal control group (receiving the vehicle only), **b)** a diabetic control group (created by the administration of Streptozotocin [STZ]), **c)** a standard drug group (administered Losartan at 10 mg/kg, orally), **d)** a group receiving Chia + Kalonji at 500 mg/kg, **e)** a group receiving Chia + Kalonji at 750 mg/kg, and **f)** a group receiving Chia + Kalonji at 1000 mg/kg. Each treatment group received its respective treatment orally once daily for six weeks. Dosage ranges for this study were chosen based on prior published studies demonstrating the safety and efficacy of Salvia hispanica (chia) and Nigella sativa (black cumin) seed extracts in rodent subjects. This experimental design allowed for evaluation of dose-dependent effects and comparison with the Nephroprotective effect of a clinically established nephroprotective agent.

Preparation of seed extracts

Validation and shade drying of the seed material from Salvia hispanica and Nigella sativa were confirmed to be performed. The seeds were ground into powder and performed successive Soxhlet extraction with ethanol (70%). The extracts obtained from the successive extraction process were concentrated using a rotary evaporator and stored at 4 degrees Celsius until ready for use. The oral formulations from the powdered seeds were prepared just prior to administration of the test agent (with 0.5% carboxymethyl-cellulose), to ensure the stability of the oral dosage form, and to maintain a uniform dispersion of both the active drug and ingoing excipients [68-72].

Biochemical parameters

The determination of parameters used to evaluate the biopharmaceutical effects of the treatments were based on analysis of selected biochemical markers present in blood collected by retro-orbital puncture under light anesthesia (at predetermined time points). Following collection of blood, serum was separated from whole blood by centrifugation (3000 RPM for 10 minutes) and analyzed to determine the concentration of selected biochemical markers. Blood glucose

concentration was determined using the glucose oxidase-peroxidase method and the HbA1c concentration was determined using ion-exchange chromatography. Renal function was evaluated by the determination of blood urea nitrogen (BUN, diacetyl monoxime method), serum creatinine (Jaffe's alkaline picrate method), and serum albumin (bromocresol green method). Therefore, the assessment of metabolic regulation and Nephroprotective outcomes was based on the evaluation of these parameters as comprehensive indicators of glycemic control and renal functional integrity **(Table 1)**.

Table 1: Glycemic indices and renal function markers in Streptozotocin-induced diabetic nephropathy rats treated with chia and kalonji seed extracts.

Parameter	Normal Control	Diabetic Control	Standard Drug	Chia+ Kalonji 500 mg/kg	Chia +Kalonji 750 mg/kg	Chia+ Kalonji 1000 mg/kg
Blood Glucose (mg/dL)	102.4 ± 3.2	552.7 ± 15.4	214.6 ± 8.7	319.8 ± 10.2	236.5 ± 9.1	166.3 ± 7.5
HbA1c (%)	4.12 ± 0.21	11.86 ± 0.35	6.21 ± 0.28	8.32 ± 0.31	6.74 ± 0.27	5.18 ± 0.25
BUN (mg/dL)	18.24 ± 1.23	69.12 ± 2.45	29.18 ± 1.67	46.30 ± 1.98	34.61 ± 1.56	23.76 ± 1.12
Creatinine (mg/dL)	0.52 ± 0.04	2.18 ± 0.12	0.86 ± 0.06	1.38 ± 0.08	0.92 ± 0.05	0.60 ± 0.04
Albumin (g/dL)	4.25 ± 0.18	2.01 ± 0.11	3.62 ± 0.15	2.87 ± 0.13	3.41 ± 0.16	4.07 ± 0.17

Oxidative stress markers (SOD, catalase, GSH, MDA)

Tissues from rats to study how the extracts of chia and kalonji seeds are able to counteract oxidative stress and also help restore antioxidant properties within the kidney. Rat kidney tissues were homogenized with a phosphate buffer (pH 7.4) to analyze oxidative stress and the antioxidant defense in the kidneys. To determine superoxide dismutase (SOD) enzyme activity, pyrogallol auto-oxidation was inhibited and catalase enzyme activity was measured using the decomposition of hydrogen peroxide. The levels of reduced glutathione (GSH) were measured using the DTNB (Elman's reagent) assay. There was also measurement of the levels of malondialdehyde (MDA) as an indicator of lipid peroxidation, which was done using the TBARS method (Thio barbituric acid reactive substances). These assays provided insight into the detrimental effects that the oxidative state has on the kidney tissue by helping identify the beneficial effects of the chia and kalonji seed extracts in restoring antioxidant capacity and reducing oxidative damage associated with diabetic nephropathy **(Table 2)**.

Table 2: Antioxidant enzyme activities and lipid peroxidation in kidney tissue of Streptozotocin-induced diabetic nephropathy rats.

Parameter	Normal Control	Diabetic Control	Standard Drug (Losartan 10 mg/kg)	Chia+ Kalonji 500 mg/kg	Chia + Kalonji 750 mg/kg	Chia + Kalonji 1000 mg/kg

SOD (U/mg protein)	28.64 ± 1.21	11.23 ± 0.87	21.35 ± 1.04	16.38 ± 0.93	20.38 ± 1.01	24.67 ± 1.12
Catalase (µmol H_2O_2 decomposed/min/mg protein)	8.23 ± 0.33	2.61 ± 0.18	6.45 ± 0.28	4.38 ± 0.22	5.73 ± 0.27	7.36 ± 0.30
GSH (µmol/g tissue)	9.82 ± 0.41	3.12 ± 0.20	7.91 ± 0.36	5.41 ± 0.27	6.76 ± 0.31	8.85 ± 0.40
MDA (nmol/mg protein)	2.15 ± 0.12	10.42 ± 0.45	4.36 ± 0.21	6.87 ± 0.29	5.12 ± 0.24	3.01 ± 0.18

Histopathological Examination

Tissue samples of the rat kidneys (i.e., 10% neutral buffered formalin) were embedded in paraffin and allowed to harden until they could be sectioned into 5 µm thick slices, and H&E (hematoxylin and eosin) staining done. The sections were then examined under light microscopy (400X magnification) for evidence of various parameters, including glomerular hypertrophy, mesangial expansion, tubular degeneration, and inflammatory infiltration. A blinded pathologist performed histological scoring to mitigate bias amongst the different samples.

Statistical analysis

Data from this study were reported as mean ± SEM of n = 6. One-way ANOVA with Tukey's multiple comparison test was the statistical analysis method used for these data. A $p < 0.05$ was the level selected for statistical significance. Graph Pad Prism 9.0 was the software used for the analysis and graphical presentation of the data.

RESULTS:

Effect on blood glucose and HbA1c

The administration of Streptozotocin caused a severe hyperglycemic state in rats but they were able to significantly improve their blood glucose and Hemoglobin A1c levels by taking either chia or kalonji extracts over time. By 500mg/kg of chia or kalonji extract, blood glucose and hemoglobin A1C levels were approximately 320mg/dL and 8.3% respectively. The 750mg/kg doses of the treatments brought the blood glucose down even further by approximately 236mg/dL and the percentage of hemoglobin A1C to about 6.7%. The administration of 1000mg/kg of chia or kalonji extracts nearly normalized blood glucose levels with the final values being approximately 166mg/dL and 5.2% respectively, and nearly equivalent to the normal control group's blood glucose and hemoglobin A1C levels. These data demonstrate a dose-dependent antidiabetic effect for both chia and kalonji extracts as shown in Figure 1.

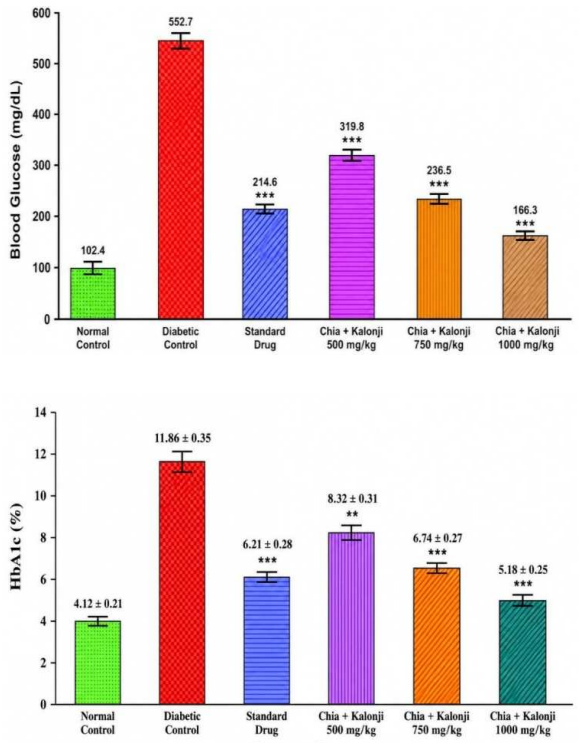


Figure 1. Blood glucose & HbA1c (%) levels in Streptozotocin-induced diabetic nephropathy rats.

Effect on renal function markers

The rats in the diabetic control group developed significant renal failure with BUN ~69mg/dL, creatinine ~2.18mg/dL, and albumin ~2.0g/dL. The administration of chia and kalonji seed extracts to the diabetic control rats significantly improved the values for all three markers of renal function. After administration of 500mg/kg of chia or kalonji seed extracts, the values for BUN, creatinine, and albumin were improved to approximately 46mg/dL, 1.38mg/dL, and 2.8g/dL respectively, as it is already shown in Figure 2. A further decrease in BUN (to ~34mg/dL) and creatinine (to ~0.92mg/dL) and a significant increase in albumin (to ~3.4g/dL) occurred for diabetic control rats receiving 750mg/kg of chia or Kalonji seed extracts. The values for BUN (~23mg/dL), creatinine (~0.60mg/dL) and albumin (~4.07g/dL) approached near-normal renal function for the diabetic control rats receiving 1000mg/kg chia or kalonji extracts.

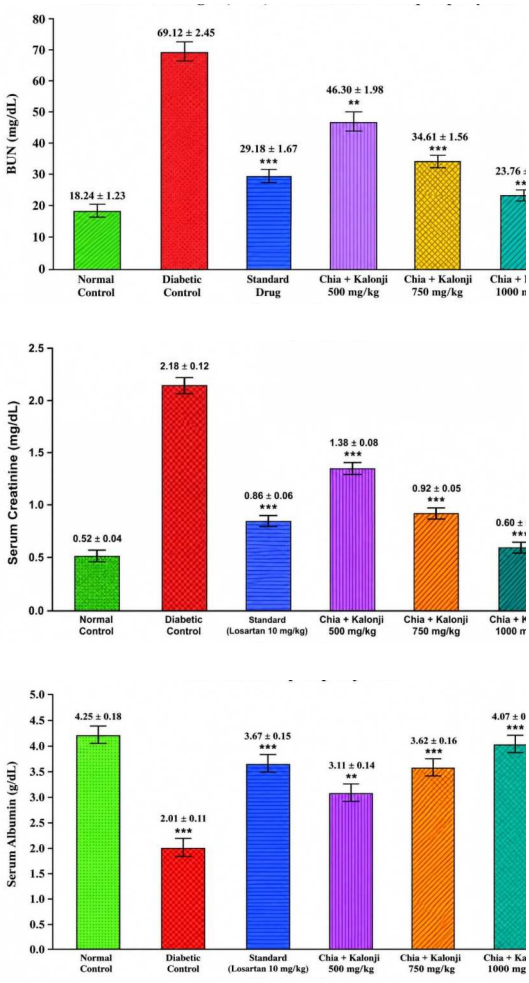


Figure 2. BUN, Serum creatinine and Serum albumin levels in diabetic nephropathy rats treated with chia and kalonji seed extracts.

Effect on antioxidant enzymes

In diabetic control rats, there was evidence of oxidative stress as indicated by reduced SOD activity (~11 U/mg protein), catalase activity (~2.6 µmol H_2O_2 decomposed/min/mg protein) and GSH levels (~3.1 µmol/g tissue). Supplementation with chia or kalonji restored antioxidant defenses in a dose-dependent manner, which is given in Figure 3. At a supplementation level of 500 mg/kg, there were moderate increases in SOD (~16 U/mg), catalase (~4.4 µmol) and GSH (~5.4 µmol). At the supplementation level of 750 mg/kg, antioxidant activity was enhanced further. At the 1000 mg/kg supplementation level, values were nearly normalized: SOD ~24.7 U/mg, catalase ~7.4 µmol, GSH ~8.9 µmol.

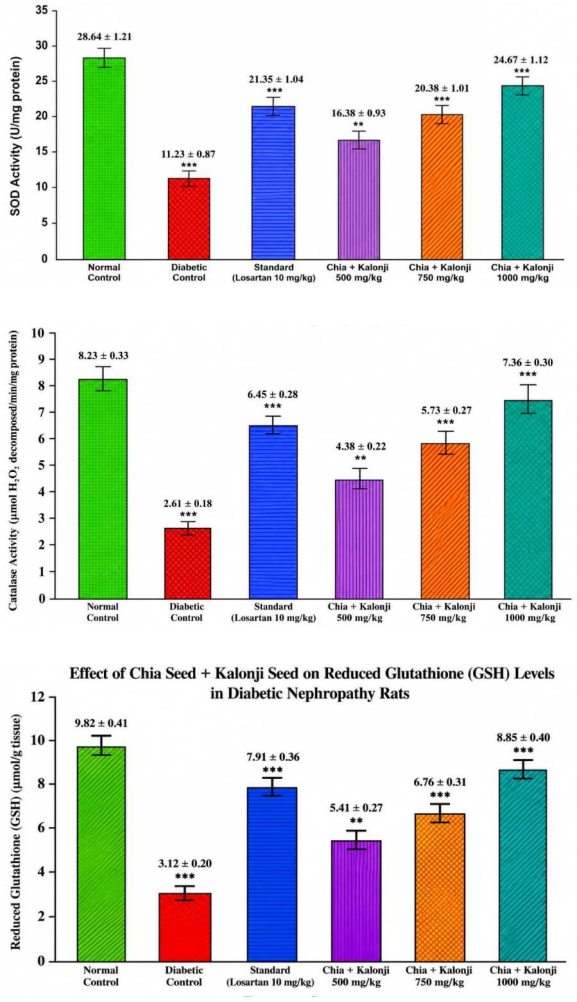


Figure 3. Antioxidant enzymes (SOD, catalase, GSH) responses in diabetic nephropathy rats following treatment with chia and kalonji seed extracts.

Histopathological findings

Histopathology of renal tissue sections of Figure 4, from diabetic control rats demonstrated glomerular hypertrophy, mesangial expansion, tubular degeneration and inflammatory cell infiltration. Rats in the standard drug group exhibited markedly enhanced renal structure; only minimal histopathologic changes were evident in the kidney tissues of these animals. Renal histology further improved in the treatment groups; there were mild improvements in renal architecture at 500 mg/kg, moderate improvements at 750 mg/kg, and nearly normal renal architecture at 1000 mg/kg. Glomeruli were structurally intact, mesangial expansion was minimal and tubular morphology resembled that of normal control rats at the highest supplementation level.

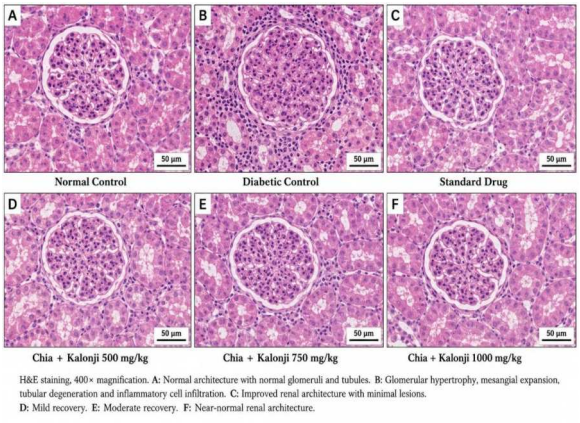


Figure 4. Representative H&E-stained kidney sections, illustrating dose-dependent recovery of renal architecture.

Dose-dependent effects

Histo-materials for each experimental variable exhibited dose-dependent efficacy of the chia and kalonji seed extracts. At the 500-mg/kg dose, the extracts conferred some protection; at the 750-mg/kg dose, substantial improvement of the histological and biochemical parameters and restoration of antioxidant status occurred. At the 1000-mg/kg dose, almost complete restoration of normal morpho functionality of kidney related-experimental parameters occurred. These results demonstrate the potential therapeutic effects of the combined chia and kalonji seeds to protect against nephron after diabetes for each of the three dose levels.

DISCUSSION

The data obtained from this experiment indicate that supplementation with *Salvia hispanica* (chia seeds) and *Nigella sativa* (black seeds) has significant antidiabetic and Nephroprotective effects in a diabetic nephropathy rat model. The reduction in fasting blood sugar and HbA1c is dose-dependent (the greater the amount of supplement given, the more the reduction), suggesting that these nutraceuticals have potential glycemic regulating properties. This finding is consistent with previous reports that chia can modulate glucose metabolism, and that kalonji helps to improve insulin sensitivity because of the thymoquinone component. Moreover, the restoration of the markers of renal function (BUN, creatinine, albumin) add further evidence to support the therapeutic use of chia and kalonji to reduce nephropathy as it relates to hyperglycemia.

The mechanism of oxidative stress is central to the pathogenesis of diabetic nephropathy and occurs as a result of an increased generation of ROS during oxidative stress, lipid peroxidation. Diabetic control rats showed a significant increase in MDA levels and a decrease in antioxidant enzyme activity, thereby demonstrating the presence of an imbalance between oxidation and antioxidants. Treatment with chia and kalonji resulted in a significant increase in SOD, catalase and GSH activity and a decrease in MDA levels. As a result of treatment, lipid peroxidation was reduced. This confirms what other researchers have shown previously, that the thymoquinone and omega-3 fatty acids act together to increase antioxidant activity and decrease inflammatory mediators. In histopathological studies, these results were confirmed through a progressive improvement in the glomerular and tubular architecture at the highest dose, with a return to normal morphology.

Chia and kalonji extracts produced improvements in both renal function and oxidative stress comparable to those achieved by Losartan, a recognized Nephroprotective agent. Thus, nutraceuticals could potentially also act as adjuncts to pharmacological therapy by providing a multi-targeted approach through antioxidant, anti-inflammatory, and glycemic pathways. The Nephroprotective effects of chia and kalonji likely occur due to chia's polyphenols and alpha-linolenic acid (omega-3 fatty acid) influencing lipid metabolism and kalonji thymoquinone suppressing reactive oxygen species (ROS) and cytokine-mediated inflammation.

These findings provide further reason to be excited about the potential for using nutraceuticals in conjunction with existing therapies for chronic

diabetic nephropathy. Current pharmacological options are limited in their ability to prevent progression to end-stage renal disease; therefore, dietary therapies including chia and kalonji may provide safe, inexpensive, and readily available options. Nevertheless, the study was limited by the following factors: preclinical design, small sample size (less than 15 patients), and no follow up greater than 12 weeks. Future studies are necessary to assess the effectiveness of chia and kalonji in humans, investigate the specific molecular pathways involved, and examine possible interactions between these herbal agents and standard pharmacological therapies.

CONCLUSION

This study demonstrates that concurrent administration of Salvia hispanica and Nigella sativa seed extract possesses significant antidiabetic and renal protective properties in diabetic nephropathy. These extracts have demonstrated enhanced glycemic regulation, improvement in renal function markers, increased antioxidant defense systems and reduced the effects of histopathology in relation to the dosage used. With the highest dosage exhibiting results comparable to traditional drug treatment, these nutraceuticals have shown a significant amount of therapeutic value. This study indicates that combining chia and kalonji seeds offers a combination feed, which could help to provide nutritional and pharmacological solutions for patients suffering from diabetic nephropathy. Additional confirmation of these findings through clinical trials will enable both seeds to be a new and cost-effective resource for the management of diabetic nephropathy and its associated complications.

Abbreviations

DN: Diabetic Nephropathy; **STZ:** Streptozotocin; **ROS:** Reactive Oxygen Species; **SOD:** Superoxide Dismutase; **GSH:** Reduced Glutathione; **MDA:** Malondialdehyde; **BUN:** Blood Urea Nitrogen; **HbA1c:** Glycated Hemoglobin; **H&E:** Hematoxylin and Eosin; **SEM:** Standard Error of Mean; **ANOVA:** Analysis of Variance; **CMC:** Carboxymethyl Cellulose; **IAEC:** Institutional Animal Ethics Committee.

Conflict of Interest: No

Funding: No

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